TOMATO BREEDING PROBLEMS IN HAWAII INVOLVING THE EFFECTS OF TOBACCO MOSAIC VIRUS AND THE INHERITANCE OF SUSCEPTIBILITY TO GREEN GEL IN THE FRUIT

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INTRODUCTION

Tobacco mosaic virus in tomato is a problem of concern and a hazard to the successful cultivation of the crop, owing to the detrimental effects of this virus on the yields. Low yields in tomato attributable to tobacco mosaic are described by McMurtrey (1929), Walter (1950, 1956), Sinclair and Brown (1958), Webber (1960) and Davis and Webb (1966).

The tomato breeding program in Hawaii, amongst other aspects has been working on the resistance to tobacco mosaic virus for over two decades. However, in recent years with the evolving of new multiple resistant varieties as described by Gilbert et al. (1961), with different levels of tolerance or susceptibility to tobacco mosaic, a closer investigation of their behaviour in relation to this virus seems necessary. Although the horticultural characters of these new varieties are described, relatively little is published on the degree of tolerance which they exhibit. These multiple resistant hybrids which have resistance to eight diseases, owe their parentage to unrelated tomato lines from Florida and Hawaii. Under the agroclimatic conditions prevalent in Hawaii, these hybrids display a tendency of green gel retention around the seeds, a character which is not commercially desirable. This phenomenon of green gel can be described as the retention of greeness around the seeds, even though the interior of the fruit turns red on ripening. This trait of green gel in some of the multiple resistant varieties grown in Hawaii has been

primarily inherited from the Florida parent STEP 305. The expression of green gel appears to be influenced both genetically and also by environment. Attempts to breed for tobacco mosaic virus resistance from this source (STEP 305) without the occurence of green gel have so far been difficult. However, whilst such a program is still under way, it would appear useful to investigate the effects of tobacco mosaic virus at different growth stages of these multiple resistant tomato varieties, together with the inheritance of susceptibility to green gel, a problem which has not been investigated hitherto as far as is known to the writer.

The objectives of the investigation envisaged are primarily to ascertain the critical stage during the growth of the tomato plant, when infection by tobacco mosaic virus will hurt it most. By a comparison of yields in the successive stages of inoculation during the growth of the tomato plants, any significant differences would indicate the economic importance of the disease in relation to the stage of infection. Also, the weight of plants above the ground level after the final harvest of the different treatments, would reveal any significant differences in the amount of growth, pertaining to the stage of infection. Simultaneously, a bioassay of the leaf samples on Nicotiana glutinosa would indicate the degree of virus concentration, in relation to the different times of inoculation.

Another aspect of the present investigation involves the study of inheritance of the gel colour surrounding the seeds of tomato. Gilbert and Acosta (unpublished) have observed the expression of both red and green gel colours, under the climatic conditions prevalent in Hawaii. Since colour is recognized as an important component and criterion of fruit quality, contributing significantly to the acceptance of both raw and processed tomato products, the practical usefulness of this aspect needs no emphasis. In this experiment, the use of varieties with green and red gel surrounding the seeds, together with their known inherent qualities for tolerance or susceptibility to tobacco mosaic virus, could possibly reveal any association between these traits.

In the year 1886, Mayer, a German botanist studied a disease of the tobacco plant which he named tobacco mosaic. He showed that the virus was infectious, but it ceased to be infective when the causal agent was exposed to a temperature of 95° C. In 1892, a Russian botanist Iwanoski confirmed some of Mayers findings, but disagreed with the others. He was able to demonstrate that the causal agent of tobacco mosaic was able to pass a bacteria proof filter. Although few workers attached any significance to this initially, Beijrinck 1898 and Baur 1904, were attempting to specify the causes of tobacco mosaic. Subsequently, controversies arose as to the exact nature of tobacco mosaic. Hunger 1905 and

Frieberg 1917 attributed it to enzymes or toxins, but Allard 1916, came to the conclusion that an organism was responsible for the disease. Meanwhile other aspects of the disease were investigated and Mulvania in 1962, showed that tobacco mosaic virus could be precipitated by protein precipitants and suspended without loosing its infectivity. This was followed by the findings of Vinson and Petre 1929, who obtained reasonably active colorless preparations of tobacco mosaic virus. They concluded that it was a nitrogen containing substance. Holmes 1929, advocated that the local lesion effect caused by tobacco mosaic virus, is correlated to the amount of virus concentration.

REVIEW OF LITERATURE

1. Color criterion (fruit interior)

Neild and Young (1966) have reported that color which is an important quality factor in tomato products, is influenced by temperature patterns prevalent during the maturity of the crop. Lycopene, the red pigment in tomatoes has been found to be limited by prolonged exposure to temperatures above 30°C or below 10°C. This pigment is responsible for the fruit color and its quality. Goodwin and Jamikorn (1952) working on the biosynthesis of carotenes as a function of time and temperature in ripening tomatoes, stated that the ripening and color formation is governed by many internal and external factors and cannot be attributed to time and tem-

perature alone. Rabourn and Quackenbush (1953) investigating the carotenes of immature and mature tomatoes, found phytoene, phytofluene and lycopene are absent in green fruits. Gilbert and Acosta (unpublished) observed genetic differences in the appearance of green gel characteristics around the seeds in tomato lines. Its expression was found not to be influenced by either nitrogen or calcium field applications in Hawaii (Waimanalo farm).

2. Symptomatology

Symptoms of tobacco mosaic fall into several categories, but a common feature is loss of color by suppression of chlorophyll development. Mogendorff (1930), describing tobacco mosaic on tomato, has stated that the incubation period is about 10 days at temperatures of 18 to 23°C. Symptom development shows three successive stages namely, stunting, malformation and mottling. In inoculation experiments with tobacco mosaic, tomato plants became mosaic in 10 to 15 days at 15°C and in 6 to 7 days at 25 to 35°C. The effect of soil temperature was apparent only in its influence on the growth of the host plant. Nichols (1952), investigating the action of certain plant hormones on the symptoms of tobacco mosaic, reported that spraying tobacco plants with alpha naphthalene acetic acid (ANA) or indole buteric acid (IBA), retarded the development of symptoms and decreased the severity of tobacco mosaic symptoms. Hare and Lucas (1960), studying the effects of pH and milk on tobacco mosaic, re-

ported that milk inactivated the virus almost completely at a pH of 6.7. Simmons et al. (1963), found that certain succulent type plants like geranium and carnation contained powerful inhibitors to tobacco mosaic virus.

Different viruses may be present together on the same host plant, like cucumber mosaic virus and tobacco mosaic virus on tomato. In such a situation, both viruses may manifest their symptoms on the same host and identification becomes difficult. The presence of tobacco mosaic can be detected by the inoculation of the extracts from such plants on Nicotiana glutinosa or Nicotiana tabacum. Even whilst employing this technique, Rappeport and Wildman (1959), have observed that there exists an intrinsic variation in sensitivity to infection on the leaf. There were areas, they reported, which were more difficult to infect with tobacco mosaic virus, together with areas which could be easily infected. The former required a greater concentration of the virus to produce symptoms than the latter. They described some areas on the leaf as entirely resistant to initial infection and those that appear least sensitive being near the petiole and leaf tip. Bancroft and Pound (1956), working on the cumulative concentration of tobacco mosaic virus in tobacco and tomato at different temperature, have reported a definite relationship between rate of host growth and the concentration of the virus. They observed, that after a maximum virus concentration was reached in the host and even

though there was an increase in the growth of the host subsequently, a decrease in virus concentration was the trend thereafter. Desjardius et al. (1954) isolated a highly virulent strain of tobacco mosaic from a virus disease complex in tomato. This strain when introduced alone into tomato, generally causes the death of the plant. Dawson (1965), observed that in susceptible tomato plants infectivity and virus particle number increased rapidly to a maximum at about 14 days after inoculation. There was less virus noticed in the leaves produced subsequently. In resistant plants, he reported that no virus was detected in the non-inoculated leaves until five weeks after the inoculation treatments. Phillip et al. (1965) studying the inheritance of resistance to tobacco mosaic in tomato, observed that with a susceptible parent symptoms appeared 14 days after inoculation, whereas in the resistant parent it took 35 to 49 days. They also stated that resistance depended on the suppression of virus multiplication within the host, basing their conclusions on virus assays made from the inoculated plants of parents and progenies.

3. Effect of Tobacco Mosaic Virus on yields of Tomato

An exception to the belief, that infection by tobacco mosaic during the early stages of growth of the tomato plant will result in low yields was found by Webber (1960). He reported that the total yield for the variety W-R-Brookston selection A, was significantly greater at the 1% level in

the treatments inoculated early, 9 days after transplanting, as compared to those inoculated about a month later. Walter (1950) studying the effect of mosaic on yield of staked tomatoes found that in the variety W-185-6, early inoculation reduced marketable yields to approximately half that for late inoculation, which was less than half that for the check plot. Alexander (1952), working on the influence of tobacco mosaic disease on the yields of unstaked tomatoes, found that delaying the time of infection reduced the loss in yields. Walter (1956), observed that in susceptible tomato plants the reduction in gross yields is not great if the infection does not occur until several fruits are well developed. He further stated, that fruits less than 3/4" in diameter at the time of infection may be reduced to two thirds its normal size, besides being inferior in color, texture and flavor to normal fruits. McRitchie and Alexander (1957), investigating the effect of certain strains of tobacco mosaic on the yields of tomato varieties, found that in three susceptible varieties namely, Rutgers, W-R-Globe and W-R-Brookston, the yields were depressed by 13%, 3% and 12% respectively. In the non-symptomatic tolerant line C. St. MV. 18 which was used as a resistant parent, all plots yielded similarly. However, when the same experiment was repeated the following summer using an inoculum obtained from a wild green type of tobacco mosaic virus on plants grown in the greenhouse, all tomato lines became infected, including the previously "resistant" line in which the yield was 38%

less than its uninoculated check. In the other three varieties mentioned earlier, the yields were reduced by 27%, 21% and 13% respectively, as compared to their check plots. Sinclair and Brown (1958), working on the effects of tobacco mosaic virus on the yields of 3 tomato varieties, found that in the variety Grothens Globe there was no significant difference in yield between the inoculated and non inoculated. Whereas in the other two varieties namely Manalucie and Moreton hybrid the non-inoculated plants gave a significantly higher yield over the inoculated. Davis and Webb (1960), working with susceptible and resistant lines of tomato have reported that in the mosaic susceptible lines, inoculation with tobacco mosaic virus reduced total fruit yields on the first 6 clusters by an average of 2 lbs. and 0.8 lb. per plant, in the fall and spring crops respectively.

4. Effect of Tobacco Mosaic Virus on internal fruit quality

Holmes (1949), Raychandhuri (1952), Boyle et al. (1957), Murakishi (1961) and Phillip et al. (1966) have observed a relationship between strains of tobacco mosaic virus and internal browning of tomatoes. Boyle and Wharton (1957), attributed internal browning to shock symptoms, resulting from the tomato fruits being invaded by tobacco mosaic virus, followed by a hypersensitive response of the host. Murakishi (1961), investigating the occurence of gray wall and internal browning disease of tomato, reported that on the varieties investigated, internal browning occured only

on plants free from tobacco mosaic virus. Both disorders he observed were worse under conditions of low light intensities. Rubatzky (1965) demonstrated a significant influence upon the appearance of internal browning attributable to tobacco mosaic. He further observed, that the highest incidence of internal browning was in red ripe fruits, followed by fruits in the pink stage. Its occurence in green fruits was reported low. Infection by tobacco mosaic was also found to significantly increase blotchy ripening in tomato. Jenkins et al. (1965), concluded by field observations and experiments that tomato fruit bronzing was clearly associated with the infection of the plant with tobacco mosaic. Phillip et al. (1966), have reported that internal browning has been demonstrated to be in part, as a consequence of late infection of the tomato plant by tobacco mosaic virus, with subsequent invasion and accumulation of the virus in the fruit.

5. Effects of nutrition on tobacco mosaic virus

Bawden and Kassanis (1954), stated that the degree of yellowing of the foliage in tomato produced by tobacco mosaic virus, depends to a great extent on the nutrition and growing conditions of the plant. It was enhanced by a lack of nitrogen, although the virus content remained less than in greener leaves with abundant nitrogen. Weathers and Pound (1954), investigating the host nutrition in relation to multiplication of tobacco mosaic virus in tobacco, found

that expressed crude sap from highest nitrogen levels were much less infectious, than extracts from lower nitrogen levels. With phosphorus, they found that increased levels had corresponding increases in virus concentration, even though the plants remained stunted. Virus production was apparently not hindered at excess levels of phosphorus, even though the growth of the plants was adversely affected. With a variation in the levels of potash that were applied, they found that the effect on virus concentration was mainly a reflection in responses of host growth. Chessin and Scott (1955), have shown that a calcium deficiency is responsible for a specific reduction in infection of Nicotiana glutinosa by tobacco mosaic virus. Shephard, Glen and Pound (1960), working with Nicotiana tabacum, reported that plants deficient in boron showed lower virus concentration for periods of one to two weeks after inoculation. Shephard and Pound (1960) observed that the virus concentration was lower in magnesium deficient plants of Nicotiana tabacum. Ling and Pound (1962) showed that the accumulation of tobacco mosaic virus in tobacco plants grown without sulphur, was distinctly and consistently less than the plants which grew in optimal sulphur levels. Garcia (1965), observed that foliar sprays of zinc appeared to be involved in virus synthesis and multiplication. A higher virus concentration was associated with the addition of zinc. Stanley (1935) suggested that the inhibitive action of zinc on tobacco

mosaic virus on Nicotiana glutinosa was not directly on the virus, but by some interaction with the host tissue. This finding was supported by Yarwood (1954), who demonstrated that 0.01% zinc sulphate decreased the local lesion appearance on N.glutinosa, but increased the local lesions on pinto bean.

Tinsley (1951), reported that well watered plants are more susceptible to virus infection than water deficient plants. Allington and Laird (1954), investigating the inhibitive effect of water on infection by tobacco mosaic, stated that dipping of rubbed leaves in water prior to inoculation, inhibited infection of Nicotiana glutinosa for a period of at least 24 hours. Pound and Welkie (1958), showed that when tobacco plants are grown in water culture with various levels of iron, they responded with a gradient of growth and characteristic deficiency symptoms. Mosaic symptoms were reduced in intensity by decreased levels of iron.

6. Transmission of tobacco mosaic virus

The common agencies for the spread of tobacco mosaic virus under field conditions are either by mechanical means, or by insect vectors with sucking or biting mouth parts. The virus often contaminates implements, clothes and hands of workers, from which it can be a source of potential dissemination. It can also spread by the rubbing of infected plants with the non infected, specially in the latter stages

of growth when the plants grow into each other. It can also be present on the seed coat of tomato, from whence the seedlings can be infected. Raychandhuri (1952), studying the retention of strains of tobacco mosaic virus in tomato seeds, reported that infection with strains of tobacco mosaic associated with internal browning, appeared only in seedlings raised from seeds which were stored for one week. The seeds that were infected with the ordinary strain of tobacco mosaic virus, retained the virus for 27 days. He also found that the dry seeds extracted from tomatoes affected by internal browning, retained the virus for a period of at least 14 days. Howles (1961), investigating the inactivation of tobacco mosaic virus in tomato seeds, reported that heating the infected seeds for 22 days at 72°C, decreased the virus concentration without adversely affecting the subsequent seedlings. It only had an affect of delaying the germination of the treated seed by 2 days. Taylor et al. (1961), working on seed transmission of tobacco mosaic virus, have reported that the site of the virus in tomato seeds from diseased plants is in and on the seed coat, with a small percentage on the endosperm, but never in the embryo. They report that the seed coat virus was eliminated by acid extraction or trisodium phosphate treatment, but not by washing in detergent solutions. The virus in the endosperm was not affected by acid or trisodium phoshate treatments, but was slowly inactivated during storage. Hoggan (1931), studying the aphid

transmission of plant viruses, stated that Myzus pseudosolani was responsible for the spread of tobacco mosaic virus in 6 different tomato varieties.

Walters (1952), demonstrated that 29% of the grasshoppers tested after an infection feeding, transmitted tobacco mosaic virus. Costa et al. (1958), have reported the transmission of tobacco mosaic virus by the agency of the adult leaf miner fly, belonging to the species Liriomyza langei Frick. They suggested that the virus is carried on the ovipositor of the insect. Broadbent (1965a) working on the transmission of tobacco mosaic virus by birds has observed that house sparrows Passer domesticus spread tobacco mosaic in tomato crops. He therefore concluded that birds could be responsible for the spread of this virus. Broadbent (1965b), investigating the tobacco mosaic virus infection through the roots of tomato plants, has stated that only a small percentage of plants became infected when grown in soil containing infected debris of tobacco mosaic virus from a previous crop. When the roots were inoculated, the tobacco mosaic virus was detected 4 to 6 months later after the experiment ceased, in the roots of many plants, but not in their shoots.

7. Breeding for resistance and genetic studies

Holmes (1943), found that hybrids between commercial tomato varieties and Lycopersicon chilense are less readily attacked by tobacco mosaic than most cultivated tomatoes. Doolittle et al. (1946), have shown that lines of Lycopersicon

hirsutum exhibited considerable resistance to common tobacco mosaic virus. Frazier and Dennett (1949), working with lines of Lycopersicon esculentum, have reported that the plum and cherry types are most resistant to tobacco mosaic. They also observed, that dominance of resistance was high working on crosses involving Lycopersicon esculentum, Lycopersicon hirsutum, Lycopersicon peruvianum and Lycopersicon pimpinellifolium. Doolittle (1955), investigating the use of wild Lycopersicon species for breeding to incorporate disease resistance, has stated that progress is hampered by factors such as certain lethal effects which make it difficult to obtain the desired combination of characters, frequent difficulty in securing the desired crosses and finally the mutable pathogen problem. Walter (1956), reported that tomato plants of a tobacco mosaic resistant selection continued to grow without symptoms after inoculation, despite the fact that the virus was present in them. However, on subsequent inoculation with tobacco etch virus, they soon developed typical mosaic symptoms. Holmes (1959), isolated a tomato line homozygous for the gene conferring resistance to tobacco mosaic, characterized by short internodes between the first 4 to 8 leaves in the seedlings. McRitchie (1957) was also able to isolate 3 strains of tobacco mosaic virus varying in their pathogenecity to tomato lines. Holmes (1960, 1961) breeding for resistance to tobacco mosaic virus in tobacco, found that the line selected for high resistance to tobacco mosaic alone,

was also a selection for resistance against 6 other viruses. He concluded that this concomitant inheritance of resistance to many virus diseases is testimony that resistance depends on the same genetic mechanism. Milinko (1962), discussing some of the breeding aspects pertaining to the control of tobacco mosaic, stated that no success was achieved in breeding a single immune or hypersensitive variety of tomato, or a Lycopersicon esculentum strain. McRitchie and Alexander (1963) investigating host specific Lycopersicon strains of tobacco mosaic, advocated that in the breeding of tomato varieties resistant to the virus, the strains of tobacco mosaic should be differentiated by their ability or inability to infect selected lines of Lycopersicon, rather than by symptom differences only. Alexander (1965), breeding tomatoes for resistance to tobacco mosaic, obtained a selection from Lycopersicon peruvianum, resistant to 5 strains of the virus.

Watson and Heinrich (1951), studying the inheritance of resistance to tobacco mosaic in interspecific crosses, obtained a ratio of 3 symptomless to one with symptoms, in the back cross with Lycopersicon hirsutum. This indicates that at least two factors are involved in symptom expression. Holmes (1952) suggested that the tendency to escape infection appeared to be due to a single dominant gene. On similar investigations, his results in 1954 showed that the findings were compatible with the supposition, that increased resistance to infection is governed by a single dominant gene.

Soost (1963) also reported that resistance to tobacco mosaic in a complex hybrid tomato appeared most likely to be governed by a single dominant gene. Walter (1956) working on the hereditary resistance to tobacco mosaic in tomato, obtained F2 ratios between resistant and susceptible stocks which indicated that parents differed in their reaction to tobacco mosaic, by 3 recessive genes. He concluded that the resistance was not similar to any of the other types described by earlier workers. Walter described this resistance as non-symptomatic tolerance, in view of the evidence that thoroughly inoculated plants contained the virus. Phillip et al. (1965) studying the inheritance of tobacco mosaic in tomato, concluded that genetically the control of inheritance to resistance is multigenic and that the behaviour of parents and progenies suggests, that resistance depended upon the suppression of virus multiplication. Cirulli and Alexander (1966) investigating the inheritance of resistance to tobacco mosaic stated that a single dominant gene in tomato is responsible for resistance to 5 strains of tobacco mosaic. Its expression they said was determined by temperature. At 60°F resistance to all 5 strains is apparent. However, between 80 and 85°F, F_1 plants were affected with mild necrosis, with strains 1, 2 and 3 and severe necrosis with strain 5. The resistance to strain 4 was not impaired at high temperatures. Continuing their work and testing a selection for resistance to five pathogenic strains at high and low

temperatures, the ratios obtained for resistance varied with both temperature and strains. Necrotic plants were heterozygous for resistance and susceptibility.

Materials and Methods

Four varieties of tomato of known behaviour in their reaction to tobacco mosaic virus were used in the experiment in which the plants were inoculated with tobacco mosaic at different growth stages. The variety Anahu shows considerable tolerance to accidental exposure to the virus under field conditions. The varieties Healani and N-52 hybrid are non symptomatic carriers of the virus, whilst STEP 174 is extremely susceptible to the disease.

ANAHU: This variety exhibits good vegetative growth, heavy and continuing yields, large fruit size, uniform ripening of fruits and a determinate growth habit. Its popularity as a variety has been enhanced by its resistance to an appreciable range of diseases namely, Fusarium wilt, grey leaf spot, a common race of spotted wilt virus, common races of root knot nematode and spider mite defoliation. Besides, it manifests a low sensitivity to infection by accidental exposure to tobacco mosaic under field conditions. It is a good combiner for use in the making of F_1 hybrids and also has the ability to recover from a poor start in the seedling stage. It has comparatively low nitrogen requirements for vegetative development and transmits this trait to its hybrids. However, besides these numerous virtues possessed by Anahu,

its drawbacks are susceptibility to vascular browning, tendency to exhibit concentric cracking under certain weather conditions, susceptibility to Alternaria leaf diseases and bacterial wilt. This was the first root knot resistant commercial type variety. It was bred by Dr. J. C. Gilbert of the Hawaii Agricultural Experiment Station prior to 1956.

HEALANI: A variety regarded as a horticultural improvement over Anahu, with a determinate growth habit and uniform ripening fruits also bred by Dr. J. C. Gilbert. The fruits are light green, with a flavor slightly better than Anahu. It has resistance to Fusarium wilt, spotted wilt virus, grey leaf spot and common races of root knot nematode. It also shows tolerance to tobacco mosaic, vascular browning, Alternaria diseases and is characterized by its ability to hold the crown set of fruits above ground level. Its disadvantage seems to be the inability to size up the fruits, specially the ones set later, under some climatic and nutritional conditions.

N-52 hybrid: An F_1 hybrid deriving its parentage from Anahu and STEP 305. Commercially it is a very popular hybrid, with an excellent, vigorous indeterminate growth habit. It produces good yields and usually emerges as the best yields and longest lived in variety trials, with the fruit size holding up remarkably well. It exhibits resistance to a number of diseases namely, Fusarium wilt, grey leaf spot, spotted wilt virus, common races of root knot nematode and

some races of leaf mold (Cladosporium fulvum). It also shows tolerance to tobacco mosaic and Alternaria diseases. The undesirable qualities attributed to this hybrid are green gel characters around the seeds under some climatic conditions and its susceptibility to concentric and irregular fruit cracking under severe cracking conditions.

STEP 174: This is a breeding line from the Charlston, South Carolina breeding laboratory, which has not yet been named or released as a variety. The plant shows remarkable vigour, besides giving good yields with large fruits. It has a semi determinate growth habit and uniform fruit ripening characters. It is resistant to Fusarium wilt and the fruits have more resistance to cracking than Anahu. Its extreme susceptibility to tobacco mosaic virus has hindered the acceptance of this breeding line for commercial purposes. Under some environmental conditions, the fruit color characteristic of green gel is seen around the seeds.

1. Procedure

Tomato nurseries for this experiment were raised in wooden flats in the greenhouse at Manoa Campus. At the age of 25 days the seedlings were transplanted at the Poamaho Experimental Station, after being dipped in a solution of Parathion to kill any insects on the plants.

The experiment took the form of a split plot design with 16 treatments, comprised of 4 varieties, 3 stages of inoculation and an uninoculated check for each variety.

The spacing was 4 ft. between the rows and 3 ft. in the row. There were 10 plants per treatment, per replicate and the treatments were replicated three times. The few vacancies that occurred were replaced three days after transplanting. A fertilizer mixture of 11:48: N.P. was applied one week after transplanting and again after the first flowers had set fruit, by placing the fertilizer on one side of each plant. The total quantity of fertilizer used for the two applications was 130 lbs. Routine weekly sprayings of Dithane and Parathion mixture at 2 lbs per 100 gallons of water each were done to control diseases and insects. All plots received furrow irrigation at biweekly intervals.

2. Inoculation: A locally prevalent common green strain of tobacco mosaic virus inoculum was obtained from tomato plants of the variety STEP 174 grown at the Manoa Campus. A 1:1 strength of the inoculum was prepared by mixing with potassium phosphate buffer solution, made up to a strength of 1/10 molar. The prepared inoculum was initially tested on Nicotiana glutinosa plants, on which it exhibited only typical necrotic lesion symptoms on the foliage, characteristic of tobacco mosaic. The inoculum was stored in the deep freeze and used to inoculate the treatments in the experiment, at 3 different growth stages namely,

(a) 2 weeks after transplanting

(b) At flowering (when the first flower buds were visible). i.e. 38 days after transplanting for

Healani, Anahu and N-52 hybrid, and 45 days after transplanting for STEP 174.

(c) At fruit set (when the first flowers set fruit) i.e., 51 days after transplanting for Healani, Anahu and N-52, and 58 days after transplanting for STEP 174.

STEP 174 came into flowering about a week later than the other three varieties and accordingly the inoculations for this variety were delayed.

The inoculations were done by rubbing the new growth of the plants in the field with the thumb and forefinger dipped into the solution of inoculum which contained carborendum. The dipping was done twice for each plant to ensure good infection. All varieties had uninoculated check plots. Three weeks after each inoculation, the new growth of the respective treatments was sampled. The inoculum from this sampling was prepared by grinding 30 gms. of the leaf tissue in a motar and pestle and mixing it with 30 cc. phosphate buffer solution. At each of these leaf samplings of the inoculated plots, the corresponding uninoculated plots were also sampled and the inoculum extracted as described above. The inoculum obtained from the different treatments was then applied onto the leaves of Nicotiana glutinosa grown in the greenhouse at Manoa Campus. Here again, the bioassay was laid out as a split plot design with four replicates. A day prior to inoculation of N. glutinosa, the excess leaves on

each plant were removed leaving four leaves of approximately equal size. The inoculation was done by rubbing the leaf surfaces with a brush dipped in the inoculum. The treatments were confined to half leaves and for each half leaf the brush was dipped twice into the inoculum. A sponge, with a piece of paper on top was held on the under surface of the leaves during inoculations. After the inoculation of each treatment, the paper over the sponge was replaced. Care was taken to see that no excess inoculum dripped onto any other leaves. Spotting was noticed on the leaves 3 to 4 days later and the counting of necrotic lesions was done after 7 days. The Nicotiana glutinosa plants for the bioassay were grown in the greenhouse and transplanted into half gallon tin cans. All plants received a fertilizer of 8:12:14 NPK one tablespoon each, a week after transplanting. Weekly sprayings with a mixture of D.D.T. and Malathion, one ounce each in two gallons of water was carried out for insect control. The plants were all healthy and dark green without blemish at inoculation.

3. Harvesting of tomatoes:

Harvesting of the fruits in the field plots at Poamoho Experiment Station was done weekly, with a total of 5 harvests commencing on the 1st August 1967 and terminating on the 31st August 1967. Great care was taken during the harvesting to prevent or minimize the spread of the virus by indiscriminate handling of the plants. This was mainly

accomplished by harvesting of the uninoculated treatments first and then moving to the inoculated plots. The fruits from each plot were harvested as marketable fruits and culls. The culls included small size fruits, together with those that were spoilt due to insect damage, disease or cracking. The weights and number were recorded for the marketable fruits. At the conclusion of the final harvest, all plants were cut at ground level and their weights recorded for each treatment.

Results:

The statistical analyses were obtained for four different data groups arising from the experiment, namely

(i) Weight of marketable fruits

(ii) Total yields i.e. marketable fruits plus culls

(iii) Weight of plants after the final harvest

(iv) Bioassay for tobacco mosaic virus concentration

The marketable fruits made up those free from any blemish due to cracking, insect or bird damage, or rotting and also excluded small size fruits. The individual data for each variety is presented in Table 1, on page 25.

1. The analysis for the weight of marketable fruits indicates,

(i) In all four varieties, the uninoculated checks have given significantly superior yields than those inoculated 2 weeks after transplanting, or at flowering, or at fruit set.

TABLE 1:

Effect of Tobacco Mosaic Virus on
Yields and Weights of Tomato Plants

Treatments	Totals of 3 replicates in lbs. (30 plants)		
	Marketable yields	Total yields	Weight of plants
1. Anahu uninoculated	115.0	173.8	47.3
2. Anahu inoculated at fruit set	109.3	178.2	54.9
3. Anahu inoculated at flowering	88.3	126.9	43.4
4. Anahu inoculated two weeks after transplanting	56.2	96.8	28.2
5. N-52 uninoculated	123.5	178.9	56.0
6. N-52 inoculated at fruit set	74.0	132.7	42.1
7. N-52 inoculated at flowering	83.5	124.7	51.4
8. N-52 inoculated two weeks after transplanting	44.5	85.7	31.0
9. STEP 174 uninoculated	88.6	125.0	44.5
10. STEP 174 inoculated at fruit set	68.6	88.9	33.1
11. STEP 174 inoculated at flowering	40.5	52.8	31.8

TABLE 1 (cont'd.)

Treatments	Totals of 3 replicates in lbs. (30 plants)		
	Marketable yields	Total yields	Weight of plants
12. STEP 174 inoculated two weeks after transplanting	23.2	35.5	14.5
13. Healani uninoculated	72.3	118.9	31.7
14. Healani inoculated at fruit set	56.4	16.0	38.5
15. Healani inoculated at flowering	25.5	71.9	33.0
16. Healani inoculated two weeks after transplanting	29.8	75.8	28.3

(ii) The inoculation with tobacco mosaic virus two weeks after transplanting has given significantly lower yields in all four varieties than the inoculations at flowering, or at fruit set, or the uninoculated check.

(iii) In all four varieties, there is no significant difference in yields whether the plants are inoculated at flowering or at fruit set.

2. The analysis for the total fruit yield reveals,

(i) For all four varieties, the total yields of the uninoculated checks and those inoculated after fruit set, are significantly greater than the treatments inoculated at flowering, or two weeks after transplanting.

(ii) There is no significant difference between the inoculations two weeks after transplanting or the inoculations at flowering for all four varieties.

3. The analysis for the weight of the plants after the final harvest indicates,

(i) For all four varieties, the weights of the plants inoculated two weeks after transplanting are significantly lower than the weights of the plants inoculated at flowering, or at fruit set, or the uninoculated check.

(ii) There is no significant difference in the weight of plants inoculated at flowering, or at fruit set, or the uninoculated check, in all four varieties.

4. The analysis on the bioassay of the leaf samples on Nicotiana glutinosa reveals,

(i) The inoculation two weeks after transplanting has given a significantly greater number of necrotic lesions in all four varieties than the inoculations at flowering, or at fruit set or the uninoculated check.

(ii) In the inoculated treatments, the susceptible variety STEP 174 has given a greater number of necrotic lesions than the other three varieties.

TABLE II:

Tobacco Mosaic Virus Content of Tomato Plants Inoculated at Different Stages of Growth As Revealed by N. glutinosa Assay

	Treatments	Total number of necrotic lesions produced on N. glutinosa from tomato plants exposed to TMV at different ages (4 replications)
1.	Anahu uninoculated	577
2.	Anahu inoculated at fruit set	319
3.	Anahu inoculated at flowering	533
4.	Anahu inoculated two wks. after transplanting	679

Table II (cont'd)

5.	N-52 uninoculated	279
6.	N-52 inoculated at fruit set	354
7.	N-52 inoculated at flowering	334
8.	N-52 inoculated two wks. after transplanting	541
9.	STEP 174 uninoculated	515
10.	STEP 174 inoculated at fruit set	826
11.	STEP 174 inoculated at flowering	863
12.	STEP 174 inoculated two wks. after transplanting	781
13.	Healani uninoculated	372
14.	Healani inoculated at fruit set	480
15.	Healani inoculated at flowering	256
16.	Healani inoculated two wks. after transplanting	534

Discussion: Considering each of the data groups for which the statistical analysis was conducted, certain findings arising out of the investigations fit in logically to one another. Dis-

cussing first, the different stages of inoculation for the analysis of the weight of good fruits alone, it appears that the inoculation two weeks after transplanting has given significantly lower yields for all four varieties, than the inoculations at flowering, or at fruit set, or the uninoculated check. This observation falls in line with the information obtained in the bioassay on Nicotiana glutinosa, which reveals that the inoculum obtained from the plants which were inoculated two weeks after transplanting,has given a significantly greater number of necrotic lesions for all four varieties than the leaf samples taken from those inoculated at flowering, or at fruit set or the uninoculated check. This may imply that a greater number of lesions indicates a higher concentration of the virus in the inoculum and consequently in the plants inoculated two weeks after transplanting. Bearing in mind that the leaf sampling for this bioassay was done three weeks after the date of inoculation, which is a total of 5 weeks after transplanting,it is obvious that this period reflects an active growing phase in the life cycle of the tomato plant. It can therefore be implied that there exists a distinct relationship between the rate of host growth and the concentration of the virus. This statement is corroborated by the findings of Bancroft and Pound (1956), working on the cumulative concentrations of tobacco mosaic virus in tomato and tobacco at different temperatures, who obtained similar results. The necrotic lesion counts for the other

treatments namely, inoculations at flowering, or at fruit set, or the uninoculated check which were significantly lower than for the inoculation two weeks after transplanting, are also in agreement with the findings of Bancroft and Pound (1956), which indicate that after a maximum virus concentration was reached in the infected plants, any subsequent growth increase in the host would give a decreased virus concentration. The implications of an early infection two weeks after transplanting, together with a greater number of necrotic lesions resulting in poor yields, is further manifested in the analysis of the plant weights after the final harvest. Here again, the plant weights of the treatments inoculated 2 weeks after transplanting are significantly lower for all four varieties, than the weights of the plants which were inoculated at flowering, or at fruit set, or the uninoculated check. It is therefore evident that an early inoculation with tobacco mosaic, two weeks after transplanting is associated with a greater concentration of the virus, together with a significantly lower fruit yield and also a significantly lower plant weight. These findings are in agreement with the results of Heubeger and Moyer (1931) and Walter (1950), who found that early infection by tobacco mosaic virus causes the greatest reduction in tomato yields. As observed in the field plots at Poamoho, the plants of the treatments inoculated two weeks after transplanting showed poor growth with sparse foliage and by no manner or means did they indicate any signs of an appreciable comeback with respect to the other inocu-

lations. This situation was clearly demonstrated in the susceptible variety STEP 174 which remained stunted, chlorotic and with malformed foliage.

Considering the treatments which were inoculated at flowering and at fruit set in the analysis for the weight of marketable fruits, there is no significant difference in yield whether the plants were inoculated at flowering or at fruit set. This can be explained on the basis of the previous finding, where there existed a distinct relationship between the rate of host growth and the concentration of the virus. Pertaining specially to tomato plants of about 4 1/2 months duration, it is reasonable to assume that once the plants reach the stage of flowering, from that point onwards its energies are diverted and harnessed mainly towards the production of fruit. It may seem that the plants have virtually restricted their actively growing vegetative phase, which possibly could act as a hindrance to unrestricted virus multiplication. These results, too, are comparable and in agreement with those of Bancroft and Pound (1956), who found a definite relationship between rate of host growth and the multiplication of the virus. Consequently, it can be inferred that after a maximum virus concentration is reached during the actively growing vegetative phase of the plant, any subsequent increase in growth on the part of the host, results in a static behaviour in relation to an appreciable multiplication of the virus. These results are further confirmed by

the statistically analyzed data arising out of the bioassay for the inoculations at flowering and fruit set, which indicate no significant difference in the lesion counts of the leaf samples of these treatments on Nicotiana glutinosa. Further, the analysis of the weight of plants recorded after the final harvest supports this finding, wherein the statistical analysis again shows no significant differences in plant weights for the treatments under discussion. For the uninoculated check plots, the statistical analysis for the weight of marketable fruits indicates a significant increase of yield over any of the inoculated plots, in all the varieties. However, due to a natural infection of the virus during the later stages of growth, the bioassay of these leaf samples on Nicotiana glutinosa, confirmed the presence of tobacco mosaic on these plants. The analysis also revealed no significant differences between these uninoculated treatments and those inoculated at flowering, or at fruit set. Here again the observations corroborate the earlier findings, whre there is a distinct association between host growth and the amount of virus present. The results of the analysis of the weight of plants further substantiates this proposition, wherein it reveals no significant differences in plant weights of the uninoculated treatments, as compared to those inoculated at flowering or at fruit set.

At this point, it would be appropriate to review the findings arising out of the statistical analysis for the

total yields which were comprised of both marketable fruits and the culls. Here the tomato yields of the uninoculated checks and those inoculated after fruit set are significantly greater than the inoculations two weeks after transplanting or at flowering. This deviation in significance where the yields from the inoculations at flowering were unable to fall in line with the treatments inoculated at fruit set, or the uninoculated checks, was primarily due to the rejection of a good percentage of these fruits owing to their small, unmarketable size. Therefore, although there was no significance between the inoculations two weeks after transplanting and the inoculation at flowering, it must necessarily be emphasized that in the former treatments non-significance was due to actual poor fruit yields pertaining to numbers, whilst in the latter instance it was as a consequence of small-sized fruits which were unable to make the grade, reflecting its quality. It would, therefore, be reasonable to suggest that infection of tobacco mosaic virus at the time of fruit set in tomato, reduces the grade of fruits in relation to commercial acceptability. This observation is in agreement with the findings of Walter (1956), who stated that the reduction in gross yield of tomato is not great, if the infection does not occur until several fruits are well developed on the plant. Considering the different tomato varieties used in this study, all aspects of the investigation indicate conclusively that the susceptible variety STEP 174

is the most severely affected by infection with tobacco mosaic virus. In the bioassay on Nicotiana glutinosa, STEP 174 has given a significantly greater number of necrotic lesions than any of the other varieties namely, Anahu, N-52 hybrid or Hea-lani. This agrees with the findings of Dawson (1965), who stated that the concentration of the virus in resistant plants remained lower than in susceptible ones. Even in the analy-ses of the plant weights and also the total yields, STEP 174 has recorded the lowest.

The interactions between varieties and inoculations pro-vide some useful information on the behaviour of susceptible and tolerant varieties towards tobacco mosaic. In so far as the susceptible variety is concerned, any infection by tobacco mosaic virus after transplanting is detrimental, as reflected by the high necrotic lesion counts in the bioassay. Discuss-ing the specific observations arising out of the bioassay, the variety STEP 174 has recorded the highest number of ne-crotic lesions in the following order.

(i)	Inoculation at flowering	863 lesions
(ii)	Inoculation at fruit set	826 lesions
(iii)	Inoculation 2 weeks after transplanting	781 lesions

There was no significant difference in the number of le-sions of the above treatments. The slightly higher lesion counts obtained in comparison to the other varieties, if it were any reflection on a small increase in the virus concen-tration of plants, can be attributed to the semi determinate

growth habit of STEP 174 which may have provided a trifle more vegetative growth during its latter stages, enhancing virus multiplication. In the case of this susceptible variety, the primary reason why the inoculation two weeks after transplanting has given slightly lower lesion counts as compared to the other two inoculations, may involve the severe shock symptoms on the young plants as a result of a rapid rate of virus multiplication. This was amply manifested under field conditions by stunted, chlorotic and malformed plants of STEP 174, which obviously could not provide any more assistance for further virus multiplication. Therefore, in a susceptible variety like STEP 174, it is reasonable to assume that unrestricted virus multiplication is prevented or hampered after the plants have reached a certain stage, when due to severe chlorosis, malformation of the foliage and stunted growth, they are no longer congenial for virus activity. On the contrary, in a tolerant variety like Anahu, there was no significant difference in the inoculation two weeks after transplanting or the uninoculated check plants, in relation to the necrotic lesion counts in the bioassay. The explanation for this occurrence may be that in varieties akin to Anahu, the tobacco mosaic virus is able to infect and multiply unhindered under field conditions. Here again, the necrotic lesions for Anahu inoculated two weeks after transplanting and the uninoculated check are greater than those obtained by inoculation at flowering, or at fruit set.

This further supports the earlier findings, where as the rate of host growth decreases, there seems to be a corresponding lull in virus multiplication. In the case of Anahu, this is more evident and applicable due to its determinate growth habit, whereby the chances for a fresh or intensified virus multiplication are very much less. The observations on the different times of inoculation and its influence as a source of natural infection on the uninoculated checks, warrants some discussion. In the bioassay conducted for the treatments which were inoculated two weeks after transplanting,the leaf samples from the uninoculated checks gave no lesions on Nicotiana glutinosa for all varieties. This implies that the check plants were free from the virus, in spite of being in the company of diseased neighbors for a period of three weeks after inoculation. Such a situation was made possible because the plants were still small and the inoculated plots had no chance of coming into contact with the checks, together with the preventive measures adopted against contamination. In the bioassay done on the leaf samples from plots inoculated at flowering, it was interesting to observe that none of the replicates from the uninoculated plots of STEP 174 had any necrotic lesions on Nicotiana glutinosa. However, in the final bioassay made from the leaf samples taken from the inoculations at fruit set, all uninoculated check plots showed necrotic lesions, with STEP 174 having the greatest number, indicating the presence of the virus in the checks due to natural infection. At this stage the

uninoculated checks had been exposed to contamination for about eight weeks. After such a length of time the prevention of natural infection is not feasible due to the vigorous growth of the plants and their foliage coming into contact in some cases, which promotes the mechanical spread of the disease. Other factors or agencies facilitating the mechanical spread would be birds, grasshoppers, etc.

Economic importance of the disease in relation to the yield of marketable fruits:

Discussing the losses in yields of marketable fruits for the variety Anahu showing tolerance to tobacco mosaic and STEP 174 a very susceptible variety, the results show a 51% loss for Anahu and a 73% loss for STEP 174, when inoculated two weeks after transplanting. For the inoculation at flowering, the variety Anahu has recorded a loss of 23% and STEP 174, 54%. In the final inoculation at fruit set, the loss for Anahu is only 4%, whilst in the susceptible variety STEP 174 it is 22%. All losses in yields are in comparison to the uninoculated checks. From these results it can be assumed that no matter whether the tomato varieties are highly susceptible to tobacco mosaic, or highly tolerant, an early infection is detrimental to marketable yields, which are reduced by over 50%. However, for a tolerant variety like Anahu, as the stage of infection is delayed, the corresponding loss in marketable yield is appreciably low, showing only a 4% loss, when infected at fruit set. The same pattern

of percentage loss in marketable yields does not hold good for a susceptible variety like STEP 174, where even the infection at a late stage like fruit set, has given a loss of 22% in comparison to the 4% recorded by Anahu under similar conditions. Tables III and IV give the general trend in percentage losses for the early inoculations. It is evident that an early infection by the virus has taken a heavy toll on all aspects of plant growth, in comparison to the later infection. It must, however, be pointed out that in the variety Healani, it has an inherent tendency of not being able to size up its large numbers of fruits under some climatic and nutritional conditions. Therefore, it has resulted in an increase in the percentage loss for fruit yields for the later infection, as compared to that two weeks after transplanting. There is also the possibility that in Healani, the inoculations at flowering and at fruit set had adverse effects on its yield, in that it aggravated this condition of its not being able to size up the fruits to a marketable grade. This may also be related to the excessive number of fruits for the size of the plant and the nutrition available to it at Poamoho Experimental Station.

TABLE III

Losses in Yields and Plant Weights of Tomato Resulting from Tobacco Mosaic Virus Inoculation Two Weeks After Transplanting

Variety	Percentage loss over uninoculated checks		
	Weight of plants	Total yields	Marketable yields
Anahu	40.38	44.30	51.13
N-52	44.64	52.10	63.97
STEP 174	67.42	72.00	73.82
Healani	10.73	36.25	58.78

TABLE IV

Losses in Yields and Plant Weights of Tomato Resulting from Tobacco Mosaic Virus Inoculation at Flowering

Variety	Percentage loss over uninoculated checks		
	Weight of plants	Total yields	Marketable yields
Anahu	8.25	26.99	23.22
N-52	8.22	30.30	32.39
STEP 174	28.54	57.76	54.29
Healani	(+)	39.53	67.73

FIGURE I. YIELD OF MARKETABLE TOMATOES IN POUNDS IN RELATION TO TIME OF INOCULATIONS.

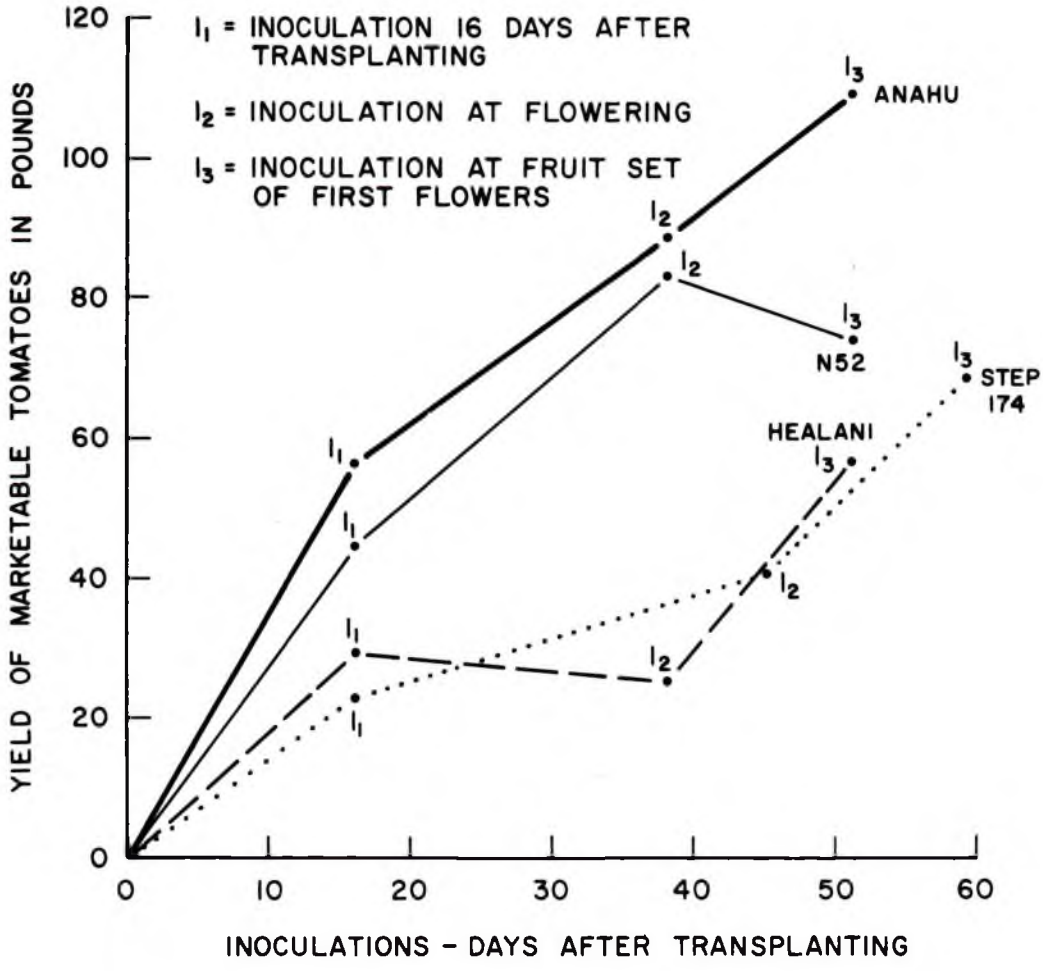


FIGURE II. TOTAL TOMATO YIELDS IN POUNDS IN RELATION TO TIME OF INOCULATIONS.

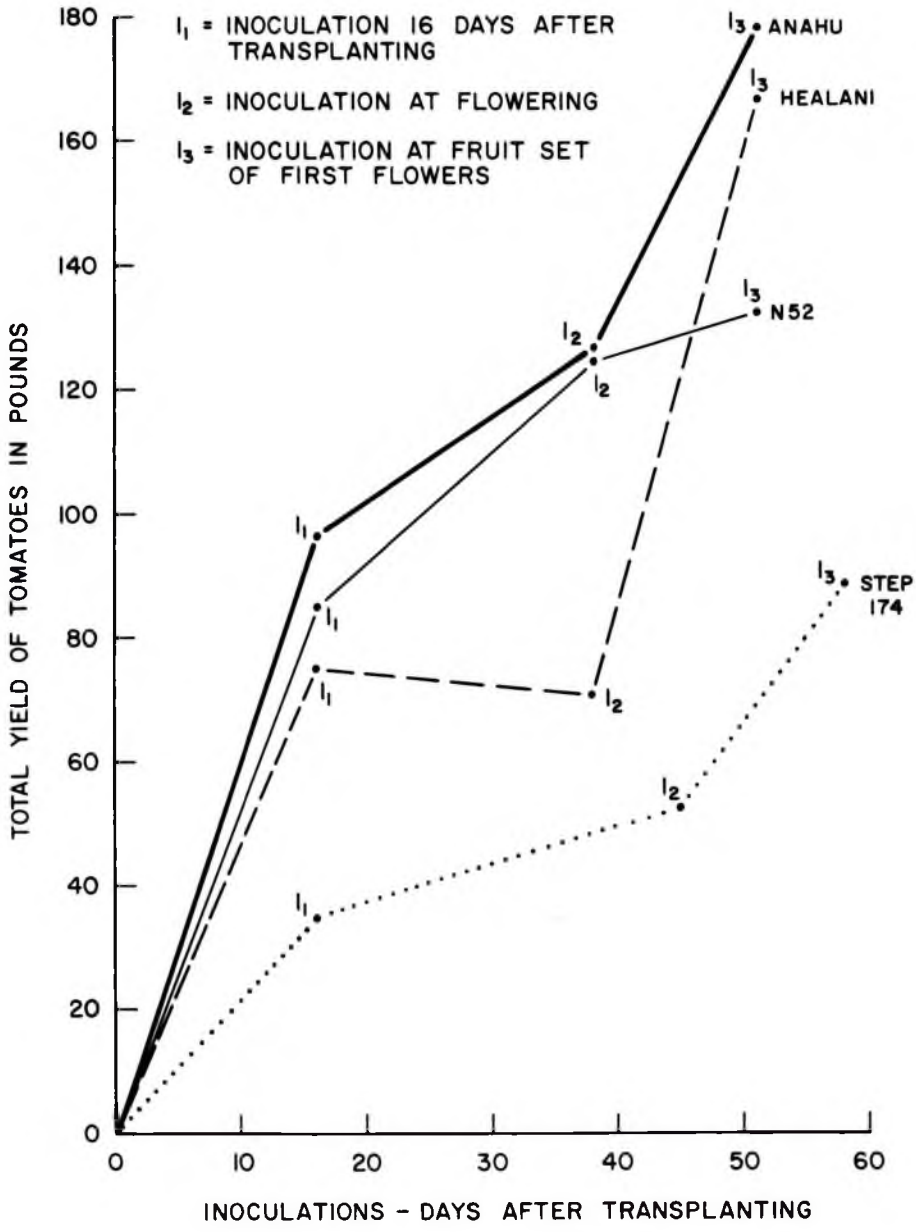
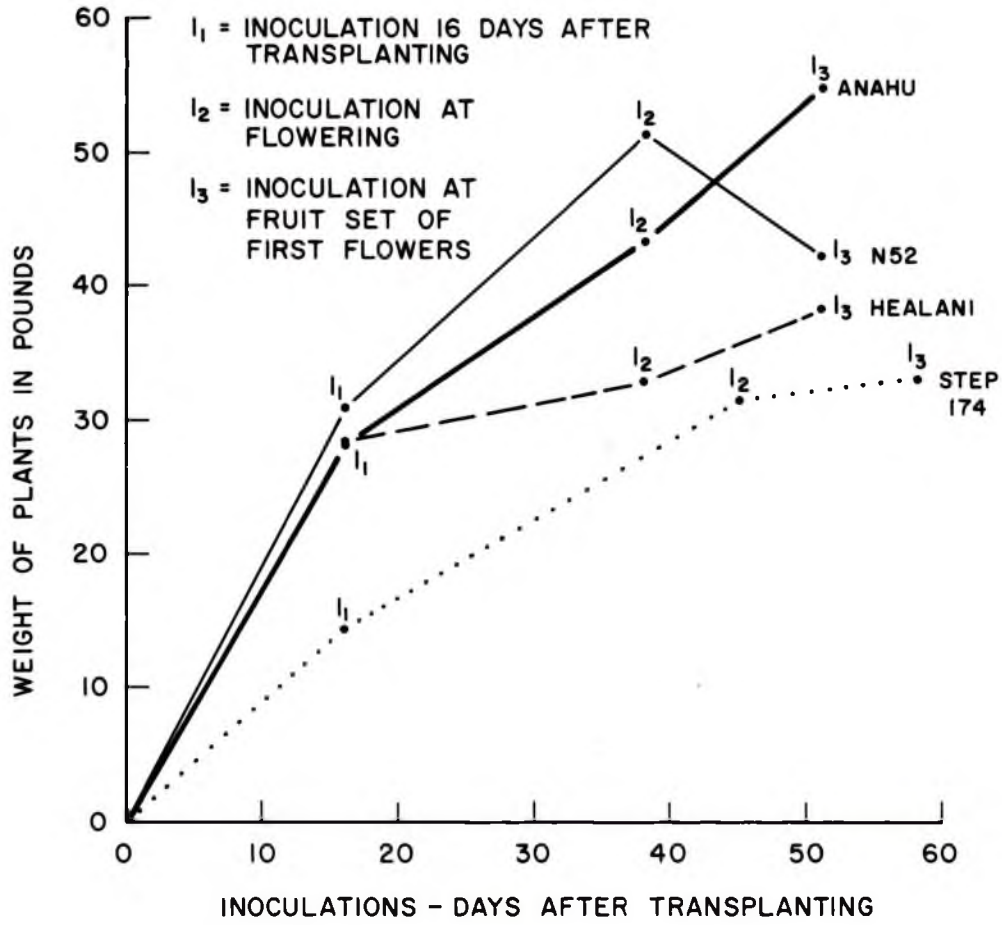


FIGURE III. WEIGHT OF TOMATO PLANTS AFTER HARVEST IN RELATION TO TIME OF INOCULATIONS.



Summary:

(i) From the economic standpoint, an early infection by tobacco mosaic virus up to two weeks from transplanting is detrimental to the marketable yields of tomato.

(ii) The infection at flowering, specially for some varieties may reduce the marketable fruits, as under such conditions the fruits may not be able to size up to the requisite grade.

(iii) Any infection by tobacco mosaic virus in tolerant varieties like Anahu after a distinct period of fruit set, is not of economic importance as far as yields are concerned, provided their harvesting period does not extend beyond a month and also that other serious disease infection does not accompany the exposure to tobacco mosaic virus.

(iv) In the bioassay on Nicotiana glutinosa, the inoculation two weeks after transplanting has given a significantly greater number of necrotic lesions than any of the other inoculations, namely at flowering, or at fruit set.

(v) The susceptible variety STEP 174, has given a greater number of necrotic lesions than any of the tolerant varieties.

(vi) There exists a distinct association between the rate of host growth and the concentration of tobacco mosaic virus.

(vii) It is evident from the bioassay, that tolerant non-symptomatic varieties are readily infected by tobacco mosaic virus under field conditions, providing congenial hosts for virus multiplication.

(viii) From the cumulative evidence arising out of the analyses for the tomato yields, plant weights and the bioassay, it appears that infection with tobacco mosaic virus two weeks after transplanting results in significantly lower fruit yields, significantly lower plant weights and also a significantly greater number of necrotic lesions on Nicotiana glutinosa. This is evidence that an early infection is hazardous to economic yields in tomato, in Hawaii.

(ix) The marketable yields under the conditions prevalent in Hawaii are reduced by over 50% if the plants are subject to an early infection, no matter whether the varieties are highly tolerant or highly susceptible to tobacco mosaic virus.

EXPERIMENT II

GEL COLOR EXPRESSION AND TOBACCO MOSAIC VIRUS STUDIES

INTRODUCTION: In this experiment the study of the occurrence of gel colors around the seeds of tomato and any association to tobacco mosaic virus infection was investigated. The gel color characters of the parents, the F_1 and the F_2 progenies were examined by comparing with the Royal Horticultural Society color chart. The tobacco mosaic virus infection occurring in each plant was classified on a scale from 1 to 5, with number one having no symptoms, to number five showing very severe symptoms.

Materials and Methods:

Four varieties of tomato, of known inherent gel color characteristics, together with their behaviour to tobacco mosaic virus were used in this study.

STEP 305: This is a breeding line from Florida, with the plant showing an indeterminate growth habit, non-uniform ripening and medium-size fruits. The fruit quality is often times marred by the green gel character it possesses. It shows good tolerance to tobacco mosaic virus. This has not yet been released as a variety.

STEP 174: A breeding line from the Charleston, South Carolina breeding laboratory, which has not yet been named or released as a variety. The plant shows remarkable vigour, besides giving good yields with large fruits. It has a semi-

determinate growth habit and uniform fruit ripening characters. It is resistant to Fusarium wilt and the fruits also show good resistance to cracking. Its extreme susceptibility to tobacco mosaic virus has hindered the acceptance of this breeding line for commercial purposes. Under some environmental conditions, the fruit color characteristic of green gel is seen around the seeds.

SC 3317. 5-1-3: This is a breeding line from South Carolina, characterized by an attractive red gel color around the seeds. The fruits have a high resistance to cracking. The plant has a determinate growth habit and is well adapted to the tropics. It is susceptible to tobacco mosaic virus.

SC 3317. 5-5-15: This, too, is a breeding line from South Carolina, with the plant showing a determinate growth habit, large fruits and an attractive red gel color around the seeds. The foliage tends to remain a light green. It is susceptible to tobacco mosaic virus.

PROCEDURE: The nurseries for the F_1 generation were raised in the green house at the Manoa Campus from already available seed, supplied by Dr. J. C. Gilbert. The following crosses and their parents were planted.

(i) SC 3317. 5-1-3 X STEP 305

(ii) SC 3317. 5-1-3 X STEP 174

(iii) SC 3317. 5-5-15 X STEP 305

(iv) SC 3317. 5-5-15 X STEP 174

(v) STEP 174

(vi) STEP 305

(vii) SC 3317. 5-1-3

(viii) SC 3317. 5-5-15

Seedlings which were 34 days old were transplanted at the Experiment Station in Waimanalo. The experiment took the form of a randomized block design with four replicates. There were five plants from each cross and also from the parents, in each replicate. The planting was done at a spacing of four feet between rows and three feet in the row. A fertilizer mixture of 14:14:14 NPK was applied two weeks after transplanting by placing four ounces of fertilizer on one side of the plant. Routine weekly sprayings of Dithane and Parathion mixture at two lbs. per 100 gallons water each were carried out for the control of diseases and insects. All plots received weekly furrow irrigation.

A total of five fruits were sampled from each plant when they had just turned red. The first sampling was done on the 20th July, i.e. 54 days after transplanting, when two fruits per plant were picked. The second sampling was done on the 4th August, i.e. 69 days after transplanting, when three fruits per plant were harvested. These fruits were cut open into halves and a few seeds from each were squeezed into a white saucer. The extraneous matter adhering to the gel was separated out by teasing the entire mass with the forefinger. The gel color immediately surrounding the seeds was then observed indoors, under good natural light conditions. The

colors were placed on a scale from 1 to 9, based on the Royal Horticultural Society color chart. They are as follows:

(i) Pea green

(ii) Pod green

(iii) Sap green

(iv) Chartreuse green

(v) Uranium green

(vi) Lemon yellow

(vii) Chinese yellow

(viii) Yellow ochre

(ix) Cadmium orange

Having examined and compared the color range for each fruit, the average color reading from five fruits for each plant was obtained. From these results, the total number of plants falling under each category on the scale was recorded. The numbers of plants falling under each color reading are represented in figure IV.

The F_2 seeds from this experiment were saved and the nurseries were planted in wooden flats at the Manoa Campus greenhouse on the 6th September 1967. Subsequently, at the age of six weeks the seedlings were transplanted at the Waimanalo Experiment Station, at a spacing of four feet between rows and three feet in the row. The seedlings were dusted with sulphur prior to uprooting to prevent damage by mites. A fertilizer mixture of 15:15:15 NPK was applied one week after transplanting, by placing four ounces of fertilizer on

one side of each plant. The experiment took the form of a randomized block design, with four replicates. Each F_2 combination had a total of 200 plants, with 50 plants per replicate. The parents had ten plants each per replicate. Routine weekly sprayings of Dithane and Parathion at two lbs. per 100 gallons water each were carried out for the control of diseases and insects. A total of five fruits were sampled from each plant when they had just turned red. The first sampling consisting of two fruits was done on the 10th January 1968 and the second sampling which comprised three fruits was done on the 19th January. The gel colors of the F_2 generation were observed by two different methods. First, the cut fruit halves were examined and placed on a scale from 1 to 5, as follows:

1. Dark green
2. Light green
3. Yellow
4. Orange
5. Red

Subsequently, the gel from each fruit was squeezed out into a white saucer and the colors matched on the Royal Horticultural Society color chart, as described for the F_1 generation. The average color readings were worked out for each plant of the different combinations and also the parents. The results are expressed in figures V, VI, VII, and VIII. Due to a spell of heavy rainfall and consequent death of some plants due to

water logging of certain areas in the experiment, it was not possible to take observations on all 200 plants of each combination. The plants which were subject to natural infection by tobacco mosaic virus, were by visual observations placed on a scale ranging from 1 to 5 as follows:

1. No symptoms
2. Very mild symptoms
3. Mild symptoms
4. Severe symptoms
5. Very severe symptoms

At each of the two samplings of the fruits, the appropriate tobacco mosaic virus reading was also recorded for each plant.

RESULTS:

The gel colors surrounding the seeds in the F_1 progeny fell into the following categories on the scale, as shown in the table below.

TABLE V

The Numbers of Tomato Plants Falling Under The Different Categories of Gel Colors in the F_1 Generation, Based on the Royal Horticultural Society Color Chart

Color Scale	Number of plants from 4 replicates: F_1			
	5-5-15 X STEP 305	5-5-15 X STEP 174	5-1-3 X STEP 305	5-1-3 X STEP 174
1. Pea green				
2. Pod green				
3. Sap green				
4. Chartreuse green	7	6	5	14
5. Uranium green	9	10	12	6
6. Lemon yellow	4	4	3	0
7. Chinese yellow				
8. Yellow ochre				
9. Cadmium orange				

The parents, STEP 305 and STEP 174 had green gel, whilst SC 3317. 5-5-15 and SC 3317. 5-1-3 had red gel surrounding their seeds.

In the F_2 population the table below gives the colors observed, when the half cut tomatoes were examined as such

and placed on a scale from 1 to 5

TABLE VI

The Numbers of Plants Falling Under the Different Categories of Gel Colors Observed in Cut Tomato Halves, Based on a 1 to 5 Rating

Color Scale	Number of plants from 4 replicates: F_2			
	5-5-15 X STEP 305	5-5-15 X STEP 174	5-1-3 X STEP 305	5-1-3 X STEP 174
1. Dark green	3	1	10	1
2. Light green	40	20	42	45
3. Yellow	21	13	24	21
4. Orange	54	64	28	72
5. Red	4	16	0	14
Total	122	114	104	153

In the other observation where the seeds were squeezed out into a white saucer and the gel colors compared on a scale ranging from 1 to 9 based on the Royal Horticultural Society color chart, the following results were obtained as shown in the table on the following page.

TABLE VII

The numbers of Tomato Plants Falling Under the Different Categories of Gel Colors in the F_2 Generation, Based on the Royal Horticultural Society Color Chart

Color Scale	Number of plants from 4 replicates: F_2			
	5-5-15 X STEP 305	5-5-15 X STEP 174	5-1-3 X STEP 305	5-1-3 X STEP 174
1. Pea green	0	0	0	0
2. Pod green	3	2	10	2
3. Sap green	16	3	28	16
4. Chartreuse green	25	17	18	30
5. Uranium green	11	4	12	7
6. Lemon yellow	19	13	16	20
7. Chinese yellow	32	27	15	27
8. Yellow ochre	15	43	5	42
9. Cadmium orange	1	5	0	9
Total	122	114	104	153

The gel colors of the parents STEP 305 and STEP 174 were green, whilst SC.3317.5-1-3 and SC.3317.5-5-15 had red gel characters. The visual observations on tobacco mosaic virus on the plants recorded on a scale of 1 to 5 are given in the table on the following page.

TABLE VIII

The Numbers of Tomato Plants Falling Into the Different Tobacco Mosaic Virus Symptom Ratings by Visual Observations

Tobacco Mosaic Virus Scale	Number of Plants from 4 replicates: F_2			
	5-5-15 X STEP 305	5-5-15 X STEP 174	5-1-3 X STEP 305	5-1-3 X STEP 174
1. No symptoms	0	0	0	0
2. Very mild symptoms	42	0	38	2
3. Mild symptoms	62	25	49	14
4. Severe symptoms	15	46	11	54
5. Very severe symptoms	3	43	6	83
Total	122	114	104	153

DISCUSSION:

The expression of gel color as shown in figure IV reveals that the F_1 progeny shows intermediate characteristics of not having green gel as green as the green parents, namely STEP 305 and STEP 174, nor does the gel color show the redness of the red parents SC 3317.5-5-15 and SC 3317.5-1-3. There appears an improvement in the green gel characteristics of the "green parents", by the hybrids displaying gel colors of a more yellowish nature. Both red gel parents, seem to

influence an improvement in the green gel characteristics of STEP 305 and STEP 174. In the F_2 populations, the green gel character of STEP 305 appears to be expressed to a greater degree than in STEP 174. On the basis of these investigations, the red gel character of SC 3317.5-5-15 seems to be better expressed in the F_2 generation than SC 3317.5-1-3, also having red gel. It appears that the inheritance of green gel is not due to a simple single gene effect.

Considering each of the F_2 combinations individually, SC 3317.5-5-15 X STEP 174 indicates that the red gel parent has given a wider spread of gel colors towards yellow and orange, in comparison with its F_1 progeny as seen in figure V. In the F_1 progeny the gel colors of the hybrids of SC. 3317.5-5-15 X STEP 174, were able to go only as far as 6 on the color scale. However, it will be seen that in the F_2 there were plants which represented points on the scale beyond 6 and way up to the end of the scale reaching 9. The greatest frequency of plants were on point 8 of the color scale.

In the F_2 combination of SC.3317.5-5-15 X STEP 305, the progeny appears to display a greater tendency towards the expression of green gel, in comparison to SC.3317.5-5-15 X STEP 174. This may indicate that the green gel characters of STEP 305 are expressed to a more appreciable degree than that of STEP 174. However, even though the number of plants showing the red gel character is negligible, there appears an

improvement in over 50% of the progeny, with the greatest frequency of plants on the 6th position in the scale, indicating a yellowish orange gel, as seen in figure VIII.

In the F_2 combination of SC.3317.5-1-3 X STEP 174, a little over 60% of the progeny show an improvement in gel color over the "green parent". As indicated earlier for the combination involving SC.3317.5-5-15 X 174, it appears from these results that the green gel character of STEP 174 is not expressed as much as that of STEP 305 in the F_2 population. In comparison to the F_1 segregation, the F_2 progeny of SC.3317.5-1-3 X STEP 174 has shown a greater number of plants with a tendency towards improvement in gel color, as shown in figure VI.

Finally, the F_2 progeny of SC.3317.5-1-3 X STEP 305 shows lower frequencies in the number of plants with improved gel color in comparison to the F_2 population of SC.3317.5-1-3 X STEP 174. Here again the results seem to fall in line with the earlier observations, where the green gel character of STEP 305 is expressed to a greater degree than that of STEP 174. In comparison with the F_1 population, the F_2 progeny of SC.3317.5-1-3 X STEP 305 shows a greater number of plants tending towards green gel. This observation may further indicate that the green gel characters of STEP 305 are likely to be expressed to a greater degree than that of STEP 174, as shown in figure VII.

SUMMARY:

Pertaining to the observations based on this experiment;

1. The green gel character of tomato line STEP 305 is expressed to a more appreciable degree in its fruits than those of STEP 174.
2. The red gel character of SC.3317.5-5-15 fruits seem to be better expressed than that of SC.3317.5-1-3, also having red gel around the seeds.
3. Both red gel parents, namely SC.3317.5-5-15 and SC.3317.5-1-3 seem to influence an improvement in the gel color of the varieties STEP 305 and STEP 174 in the F_1 and F_2 progenies.
4. The F_1 progeny shows intermediate characters of not having green gel as green as the "green" parents, nor red gel as red as the "red" parents.
5. It appears that the inheritance of green gel is not due to a simple single gene effect.
6. There appears no association between the occurrence of gel color and tobacco mosaic virus infection, as far as observed in these trials.

FIGURE IV. GEL COLOUR SURROUNDING THE SEEDS OF TOMATO - F_1 GENERATION.

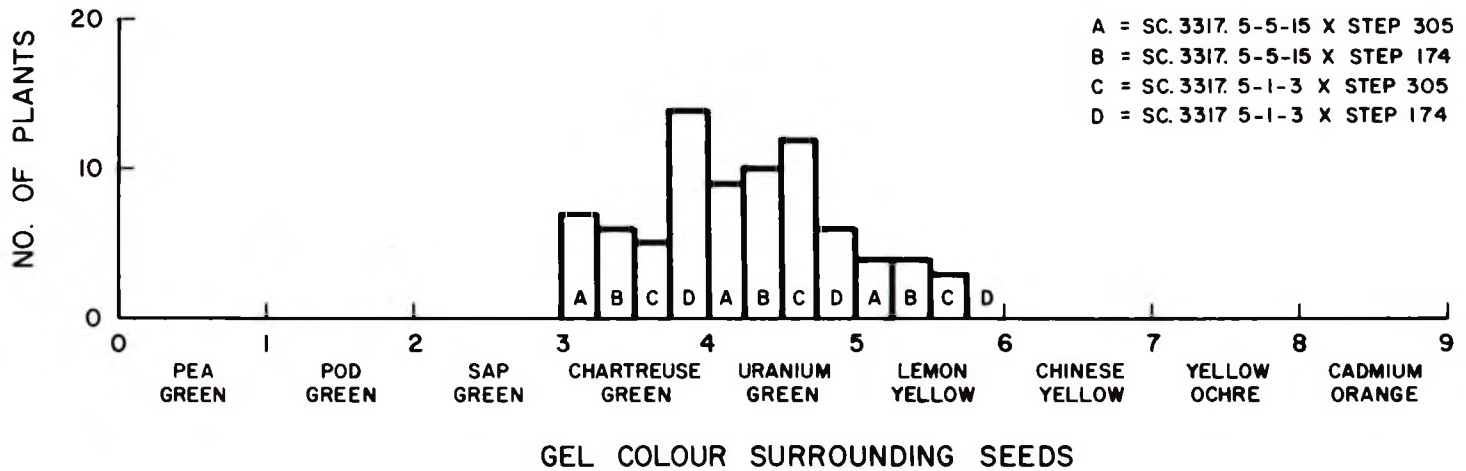


FIGURE V. GEL COLOUR SURROUNDING THE SEEDS OF TOMATO - F_2 GENERATION.

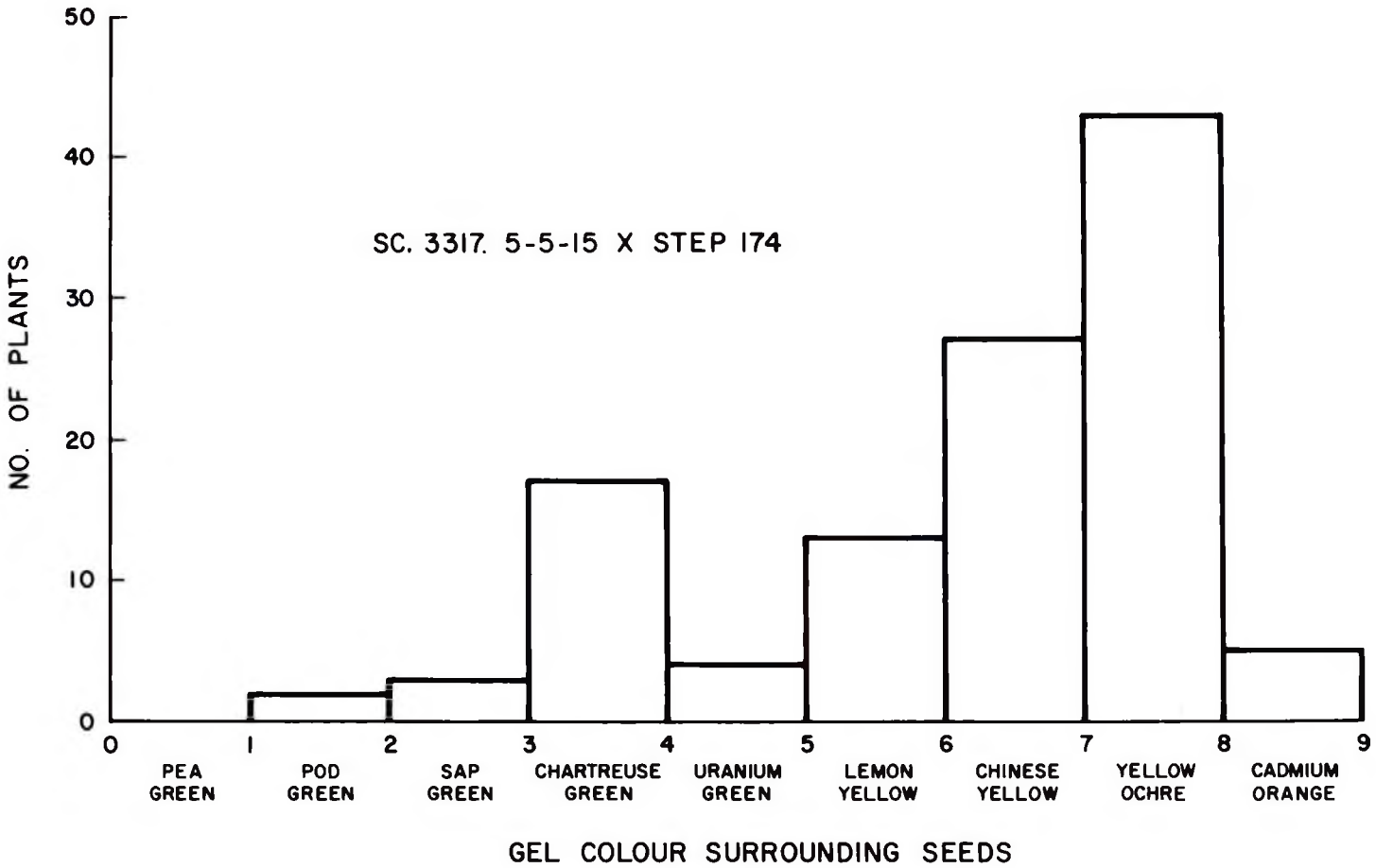


FIGURE VI. GEL COLOUR SURROUNDING THE SEEDS OF TOMATO - F_2 GENERATION.

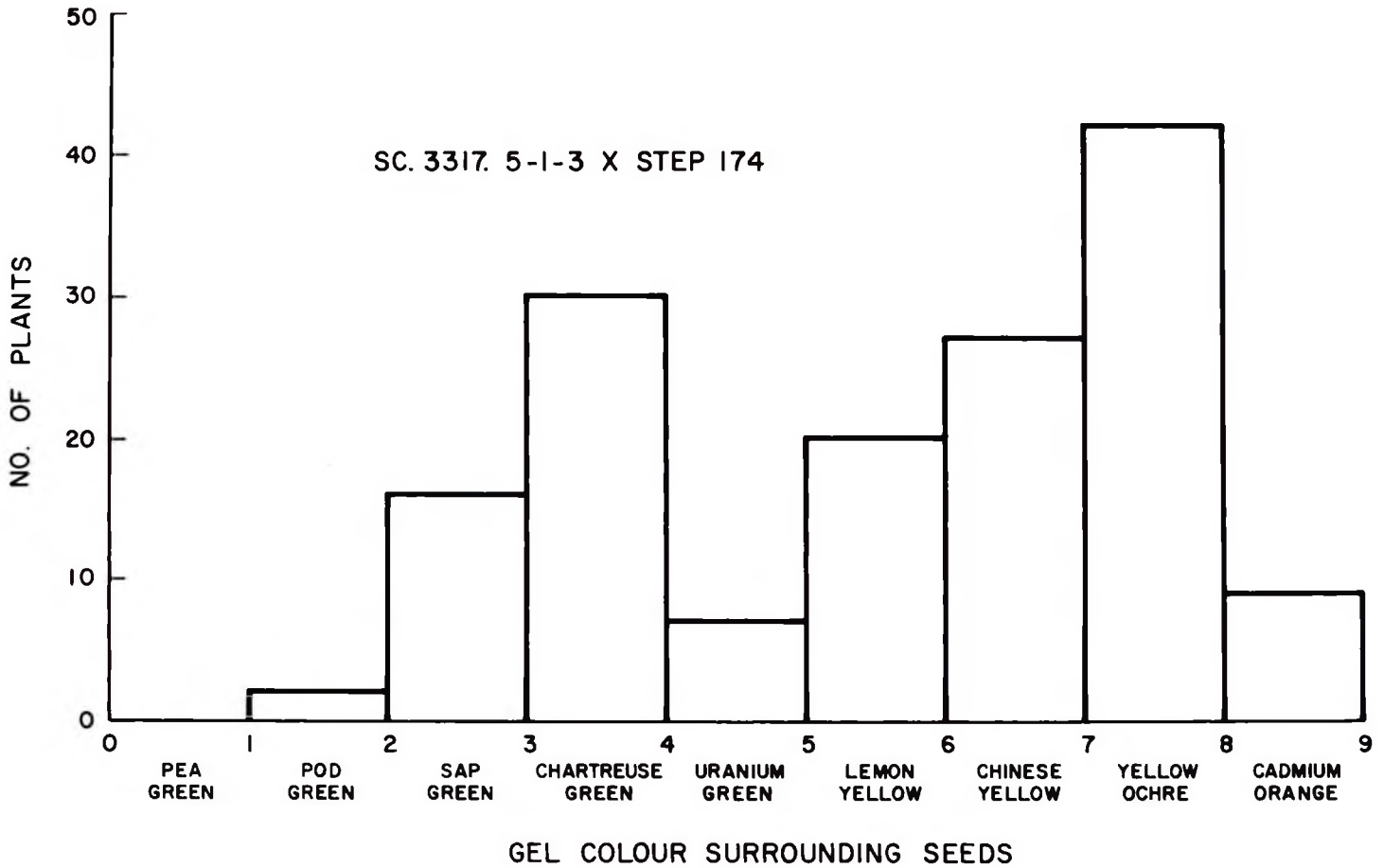


FIGURE VII. GEL COLOUR SURROUNDING THE SEEDS OF TOMATO - F_2 GENERATION.

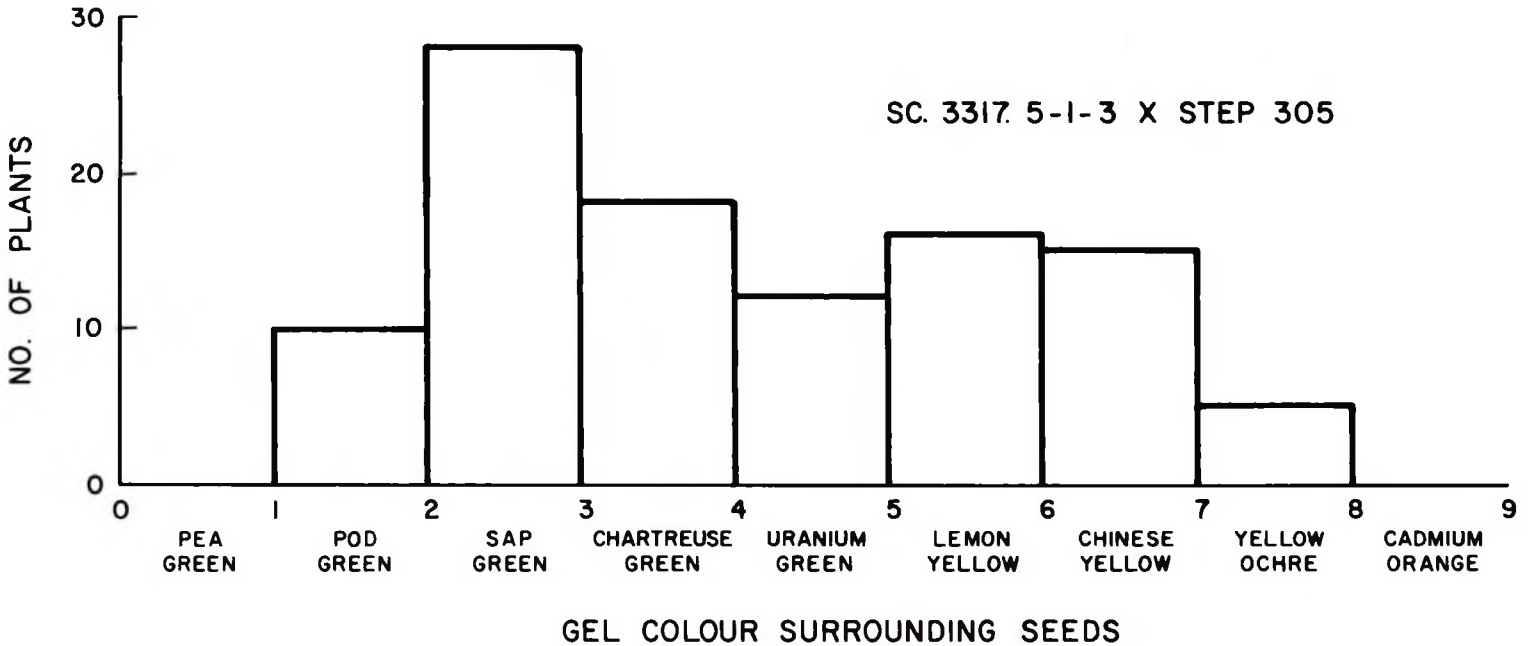
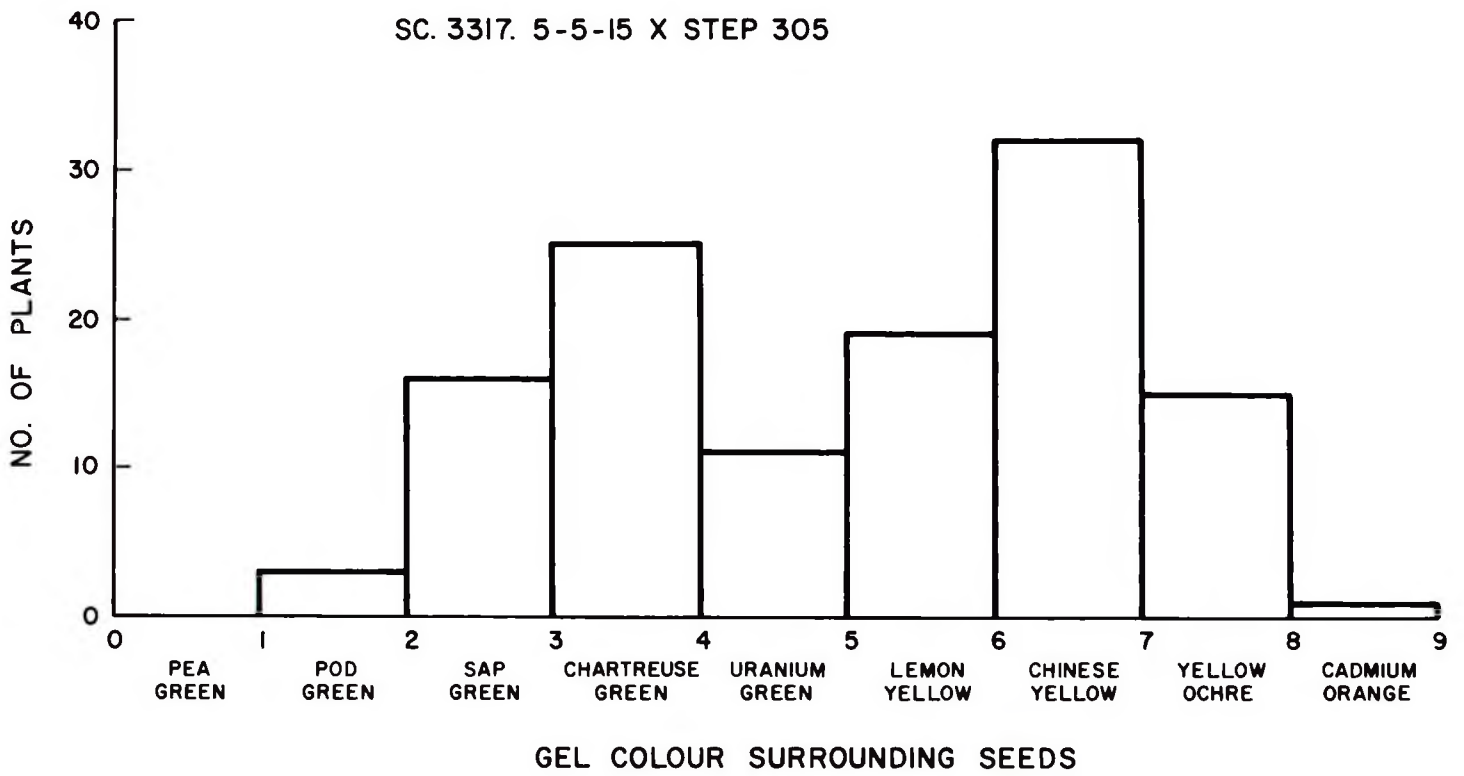


FIGURE VIII. GEL COLOUR SURROUNDING THE SEEDS OF TOMATO - F_2 GENERATION.



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